Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6070–6074, September 1985
Biochemistry

Transcription of the human *hsp70* gene is induced by serum stimulation

(heat shock/transcriptional regulation/DNA synthesis/human cells)

BARBARA J. WU AND RICHARD I. MORIMOTO*

Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60201

Communicated by Phillip A. Sharp, May 20, 1985

ABSTRACT **We have examined the expression of the heat shock protein (*hsp70*) gene in human cells. The transcription of the *hsp70* gene and accumulation of cytoplasmic hsp70 mRNA is induced by serum stimulation. Populations of HeLa cells and human embryonic kidney cells (cell line 293) were serum starved. Upon serum stimulation, the level of hsp70 mRNA transiently increases between 12 and 18 hr to a 10-fold higher level. The increased levels of hsp70 mRNA can be accounted for by a 10- to 15-fold increase in the rate of transcription of the *hsp70* gene. When cells were serum-stimulated in the presence of an inhibitor of DNA synthesis, 1-β-D-arabinofuranosylcytosine (araC), the levels of hsp70 mRNA were induced to only 20% of the maximal level detected in the absence of the inhibitor. This suggests that the expression of the *hsp70* gene is coupled to DNA synthesis. The cloned human *hsp70* gene contains regulatory sequences that confer serum-stimulated transcriptional control. The endogenous *hsp70* gene and the transfected chimeric gene containing sequences upstream of the *hsp70* gene fused to bacterial chloramphenicol acetyltransferase are both temporally expressed in stable transfectants of cell line 293 cells. The endogenous hsp70 mRNA and the chimeric mRNA reach maximum levels 12–18 hr after serum stimulation.**

The recent discoveries of different functional classes of viral oncogenes and their cellular counterparts, the protooncogenes, have provided insights into the events that lead to cellular transformation. One class of viral oncogenes that does not appear to have cellular analogs is the transforming genes of DNA tumor viruses. The process of DNA tumor virus-initiated cellular transformation can be revealed by identifying cellular genes whose expression is directly affected by viral transforming gene products. The transforming gene products of adenovirus serotype 5 (Ad5) E1A and polyoma large T antigen have been implicated in the immortalization of primary cells in culture (1–6). The mechanism(s) by which primary cells become immortalized is not understood. Yet, it would seem likely that some of the events are mediated through altered expression of cellular genes. Indeed, adenovirus E1A has been shown to be both a positive transcription activator (7–11) and a repressor (12, 13) of viral and cellular gene expression. One cellular gene that is transcriptionally activated by two different oncogene products, adenovirus E1A (14, 15) and mouse rearranged c-myc (16), is the gene that encodes the major 70,000-Da heat shock protein (*hsp70*).

We have isolated a human *hsp70* gene that is induced early in Ad5 infection of HeLa cells and is constitutively expressed in Ad5-transformed human embryonic kidney cells (cell line 293 cells) (17). The high levels of hsp70 mRNA in cell line 293 cells was expected because these cells express the Ad5 *E1A* gene (18), whose product has been shown to induce the synthesis of hsp70. HeLa cells also express *hsp70* at low but detectable levels (15, 17). The constitutive synthesis of hsp70 mRNA in HeLa and cell line 293 cells maintained at normal growth temperatures suggests that the expression of the *hsp70* gene can be regulated by mechanisms independent of heat shock.

In this study, we show that transcription of the *hsp70* gene and levels of hsp70 mRNA are induced by serum stimulation in two human cell lines, HeLa and 293 cells. The increase in hsp70 mRNA after serum stimulation is blocked by 1-β-D-arabinofuranosylcytosine (araC), an inhibitor of DNA synthesis. The DNA sequences that respond to serum-stimulated expression reside in the 5′ upstream regions. A chimeric gene, containing the heat shock promoter fused to the bacterial gene encoding chloramphenicol acetyltransferase, retains the serum-stimulated expression observed for the endogenous *hsp70* gene.

MATERIALS AND METHODS

Cells and Plasmids. HeLa and 293 cells (from P. Sharp, Massachusetts Institute of Technology) were grown in Dulbecco's modified Eagle's medium (DME medium) with 5% fetal calf serum at 37°C. Plasmid pH2.3 [a 2.3-kilobase (kb) *Bam*HI/*Hin*dIII fragment] contains the entire *hsp70* coding region (17). Plasmid pHBCAT, constructed by R. Kingston, contains 2.4 kb of 5′ sequences of the human *hsp70* gene fused with the bacterial chloramphenicol acetyltransferase (*CAT*) gene (17). Plasmid pSV2-ECOGPT contains the bacterial xanthine/guanine phosphoribosyltransferase gene (19). Plasmid pJOL contains 0–7.8 map units of Ad5 (20).

Isolation of RNA, S1 Nuclease Protection, and Run-on Transcription. Cytoplasmic RNAs were isolated and the levels of hsp70 mRNA were determined by S1 nuclease protection using plasmid pH2.3 3′-end-labeled at the *Bam*HI site (17). The S1 nuclease-protected fragments were analyzed by alkaline/agarose or polyacrylamide gel electrophoresis. The relative amounts of hsp70 mRNA were established by quantitation of densitometric scans of the autoradiographic exposures or by liquid scintillation counting of the excised S1 nuclease-protected fragments.

Nuclei were isolated from cells in 10 mM Tris·HCl, pH 7.4/10 mM NaCl/3 mM $MgCl_2$/0.5% Nonidet P-40. The nuclear pellet was frozen in an equal vol of 40% glycerol/50 mM Tris·HCl, pH 8.3/5 mM $MgCl_2$/0.1 mM EDTA at 2×10^9 nuclei per ml. [^{32}P]UTP-labeled nascent transcripts were prepared by *in vitro* runoff transcription using isolated nuclei and were hybridized to plasmid DNAs (21).

Serum Stimulation. HeLa and 293 cells were plated in DME medium containing 5% fetal calf serum at 10% confluence and

Abbreviations: hsp, heat shock protein; araC, 1-β-D-arabinofuranosylcytosine; Ad5, adenovirus serotype 5; kb, kilobase(s); CAT, chloramphenicol acetyltransferase.

*To whom reprint requests should be addressed at: Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, 2153 Sheridan Road, Evanston, IL 60201.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

grown to 80% confluence. The cells were washed with warmed phosphate-buffered saline, incubated for 48 hr in DME medium without serum, and stimulated with fresh medium containing 20% fetal calf serum. At the indicated times after stimulation, the cells were processed and nuclei and cytoplasmic RNA were isolated.

Suspension cultures of 293 cells were maintained at 2×10^5 cells per ml in Joklik's medium containing 5% fetal calf serum. For serum starvation, the cells were grown to a density of 6×10^5 cells per ml, collected by centrifugation, and returned to Joklik's medium containing 0.5% fetal calf serum for 72 hr. The cells were stimulated by addition of 20% fetal calf serum. At the indicated times, 1-ml aliquots were labeled with [^{3}H]thymidine (2 μCi/ml; 1 Ci = 37 GBq), lysed in 0.1 M NaCl/10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.5% $NaDodSO_4$, diluted 1:3.5 with H_2O and brought to a final concentration of 10% (vol/vol) trichloroacetic acid.

DNA Transfection and Colony Selection. Cell line 293 cells were cotransfected with 10 μg each of pHBCAT and pSV2-ECOGPT. Cells were plated at a density of 10^6 cells per 10-cm^2 dish and were incubated for 24 hr with DNA–calcium phosphate coprecipitates (22). Cells were washed twice with warmed phosphate-buffered saline, and fresh medium was added. After 2 days, the cells were trypsinized and plated at 10^5 cells per 10-cm^2 dish and incubated in DME medium containing 25 μg of mycophenolic acid per ml (Eli Lilly), 250 μg of xanthine per ml, 10 μg of thymidine per ml, 25 μg of hypoxanthine per ml, 2 μg of aminopterin per ml, 150 μg of L-glutamine per ml, and 5% fetal calf serum (19). Approximately 10–20 colonies per dish were visible by day 10, and individual clones were isolated and expanded.

RESULTS

Expression of the *hsp70* Gene in Serum-Stimulated Cells. We examined the levels of cytoplasmic hsp70 mRNA in cells that were serum-starved and subsequently serum-stimulated. Cells maintained in the serum-deficient medium for 2 days were stimulated by addition of fresh medium containing 20% fetal calf serum. We detected a marked decrease in the growth rate of cell line 293 cells in serum-deficient medium. The lack of cell growth over the 2-day period did not affect cell viability.

The level of hsp70 mRNA was measured by S1 nuclease protection, using a ^{32}P-labeled template prepared from the cloned human *hsp70* gene and an equal quantity of cytoplasmic RNA from each time point after serum stimulation. The S1 nuclease-resistant products were analyzed by gel electrophoresis (Fig. 1). Cells maintained in serum-deficient medium contain lower levels of hsp70 mRNA than in exponentially growing cells (data not shown). In cell line 293 cells, the low level of hsp70 mRNA is maintained until 6–8 hr after serum stimulation. The level of hsp70 mRNA increases 10-fold at 12–18 hr and is reduced to 30% of maximum by 22 hr. Neither the size of the hsp70 transcript nor the site of transcription initiation is altered in serum-starved or in serum-stimulated cells (data not shown).

We were concerned that the results on the expression of the *hsp70* gene in cell line 293 cells might be unique. Therefore, we examined the expression of the *hsp70* gene in HeLa cells using the same regimen of serum starvation and serum stimulation. The temporal pattern of hsp70 mRNA accumulation in serum-stimulated HeLa cells is similar to that observed for cell line 293 cells (Fig. 2). The level of hsp70 mRNA in HeLa cells increases 10-fold by 12–18 hr after serum stimulation and declines to lower levels by 30 hr. The similar results obtained using either cell line 293 (Fig. 1) or HeLa cells (Fig. 2) suggest that serum-stimulated regulation of *hsp70* gene expression is an intrinsic property of the *hsp70* gene.

Transcription of the *hsp70* Gene in Serum-Stimulated Cell Line 293 Cells. The accumulation of higher levels of hsp70

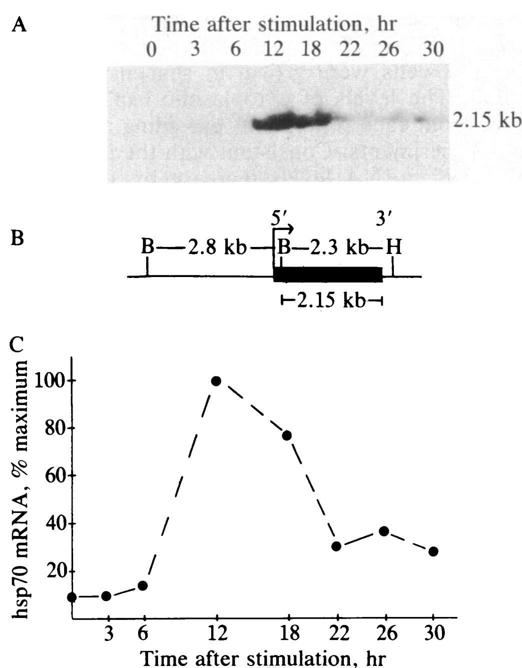

FIG. 1. The levels of cytoplasmic hsp70 mRNA in serum-stimulated cell line 293 cells. (*A*) Relative levels of cytoplasmic hsp70 mRNA were determined by S1 nuclease protection using pH2.3 as template (12-hr exposure). (*B*) Physical map of the human *hsp70* gene (solid area) and the direction of transcription (arrow). The 2.8-kb *Bam*HI fragment contains 150 base pairs of 5′ nontranslated sequences and upstream sequences. B, *Bam*HI; H, *Hin*dIII. (*C*) Relative levels of hsp70 mRNA in stimulated cells. The autoradiogram in *A* was quantitated and results were normalized to the maximum level of hsp70 mRNA (12 hr).

mRNA after serum stimulation could be due either to an increase in the rate of transcription of the *hsp70* gene or to altered stability of hsp70 mRNA. We examined the former possibility by measuring the relative rates of transcription of the *hsp70* gene in serum-starved and subsequently in serum-stimulated cell line 293 cells. We used the method of run-on *in vitro* transcription in isolated nuclei in which preinitiated

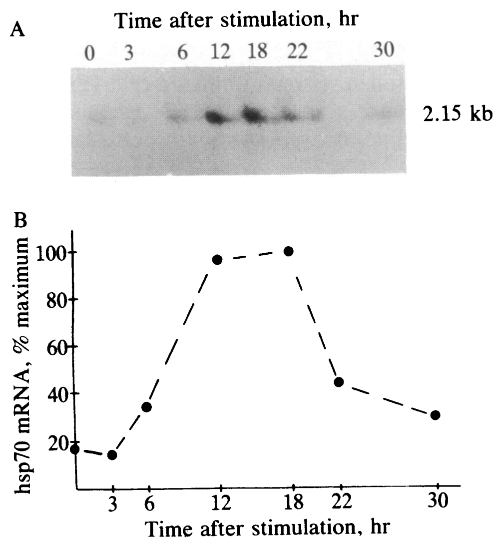

FIG. 2. Levels of cytoplasmic hsp70 mRNAs in serum-stimulated HeLa cells. (*A*) Relative levels of cytoplasmic hsp70 mRNAs were determined by S1 nuclease protection (as described in Fig. 1) (48-hr exposure). (*B*) Relative levels of hsp70 mRNA in serum-stimulated cells. The autoradiogram in *A* was quantitated as described in Fig. 1.

nascent transcripts are elongated in a reaction cocktail containing [^{32}P]UTP. To obtain a sufficient quantity of nuclei, cell line 293 cells were grown in suspension and serum-stimulated. The levels of cytoplasmic hsp70 mRNA were measured from each time point, providing a comparison to previous experiments. Consistent with the results shown in Fig. 1, hsp70 mRNA levels increase by 10 hr and reach maximum levels by 18 hr after stimulation (Fig. 3*A*). The ^{32}P-labeled nascent transcripts isolated from nuclei of serum-stimulated cells were hybridized to nitrocellulose filters containing plasmid DNAs for the vector PAT153, the cloned *hsp70* gene (pH2.3), and the cloned adenovirus *E1A* and *E1B* genes (pJOL). Plasmid pJOL provides an internal control to which the transcriptional activity of the *hsp70* gene can be compared. Transcription of the *hsp70* gene increases 10- to 15-fold 10–18 hr after serum stimulation (Fig. 3 *B* and *C*). For comparison, the rate of transcription in heat shocked cells increases 15-fold after 15 min at 43°C. Whereas hsp70 mRNA declines to undetectable levels at 30 hr, the *hsp70* gene continues to be transcribed at 30% maximal rates. We conclude that the increase in hsp70 transcription can account for the increase in levels of cytoplasmic hsp70 mRNA in serum-stimulated cell line 293 cells.

In contrast, transcription of the integrated *E1A* and *E1B* genes does not display the same temporal pattern observed for the *hsp70* gene. Whereas the *hsp70* gene is not transcribed in serum-starved cells, substantial levels of hybridization to pJOL are detected (Fig. 3*B*). Transcription from *E1A* and *E1B* genes appears to be temporally regulated after the addition of serum; however, our studies do not distinguish which pre-early genes are transcribed. Indeed, by 18 hr when transcription of the *hsp70* gene is at maximal levels, transcription of Ad5 *E1A* and *E1B* has declined.

DNA Synthesis and *hsp70* Gene Expression. In previous experiments, we had observed a temporal relationship between *hsp70* gene expression and DNA synthesis. To examine this relationship, cell line 293 cells were serum-starved and subsequently serum-stimulated in the presence of the DNA synthesis inhibitor araC (23). Two populations of cells were maintained—control and araC treated. At various times after serum stimulation, aliquots were removed, [^{3}H]thymidine incorporation was measured, and the level of hsp70 mRNA was determined by S1 nuclease analysis. In the presence of araC, 90% of the serum-stimulated DNA synthesis was blocked (Fig. 4*A*). The araC treatment also depressed the level of hsp70 mRNA to only 20% of the maximum detected in serum-stimulated cells (Fig. 4 *B* and *C*). The araC block appears to be specific because neither the level of [^{3}H]uridine incorporation into pulse-labeled nuclear RNA nor the steady-state level of α-tubulin mRNA was affected (data not shown). Although the expression of the *hsp70* gene is no longer serum-stimulated in the araC-treated cells, it remains heat shock inducible. Cells serum-stimulated and araC-treated for 16 hr were heat shocked, and the level of hsp70 mRNA was measured by S1 nuclease analysis. As shown in Fig. 4*B*, the level of hsp70 mRNA is induced 12-fold by heat shock. This heat shock induction of hsp70 mRNA is much greater than the normal 2-fold heat shock induction in exponentially growing cell line 293 cells (17). However, serum-starved cells have a lower basal level of hsp70 mRNA. Indeed, the levels of hsp70 mRNA attained upon heat shock of the araC-treated cell line 293 cells and exponentially growing 293 cells are comparable. Treatment of 293 cells with araC does not alter either overall RNA metabolism or the heat shock-induced transcriptional machinery. We conclude that DNA synthesis and hsp70 mRNA levels are coupled.

Serum-Stimulated Expression of the Chimeric Heat Shock *CAT* Gene. We have previously demonstrated that the nucleotide sequences upstream of the 5′ terminus of the *hsp70* gene confer both heat shock and Ad5 inducibility (17). To determine whether this region also contains sequences responsive to serum stimulation, we generated stable transfectants of cell line 293 cells containing a chimeric gene, pHB-CAT. This chimera is composed of 2.6 kb of upstream sequences from the genomic human *hsp70* gene fused to a heterologous test gene encoding bacterial CAT.

Cell line 293 cells were cotransfected with pHB-CAT and pSV2-ECO-GPT and were selected on medium containing mycophenolic acid (22). Six independent clones were isolated and expanded, and cytoplasmic RNAs were prepared from cells maintained at 37°C or heat shocked at 43°C. The pHB *CAT* gene was constitutively expressed at 37°C in each of the six independent transformants. The level of pHB-CAT mRNA increased 2-fold after heat shock, as does the endogenous hsp70 mRNA (Fig. 5). Each of the transformants was serum-starved and serum-stimulated to determine whether the expression of the heterologous gene mimics the endogenous *hsp70* gene. The results for one clonal line (293-pSV2-HB-CAT) are shown. The endogenous hsp70 mRNA and the heterologous pHB-CAT mRNA in serum-deprived and serum-stimulated 293-pSV2-HBCAT-12 cells were measured by S1 nuclease protection. Specific DNA templates that distinguished between the endogenous *hsp70* gene and pHB-CAT mRNA were prepared. The size of S1 nuclease-protected fragments are 2.15 kb for the endogenous hsp70 mRNA and 400 base pairs for the pHB-CAT mRNA (17). The similar temporal pattern of pHB-CAT and hsp70 mRNAs shown by clone 293-pSV2-HBCAT-12 (Fig. 5 *C* and *E*) was

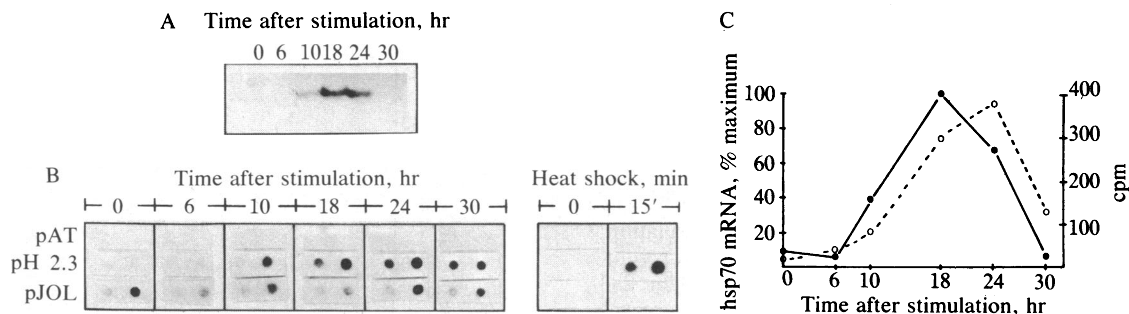

FIG. 3. Transcription of the *hsp70* gene in serum-stimulated cell line 293 cells. (*A*) Relative levels of cytoplasmic hsp70 mRNAs were determined by S1 nuclease protection (12-hr exposure). (*B*) Nuclei were prepared from cells at the indicated times (hours) after serum stimulation. [^{32}P]UTP-labeled *in vitro* elongated run-on transcripts prepared from 10^8 cells were hybridized to nitrocellulose filters containing 0.25 μg and 1 μg of plasmids: pAT153, human *hsp70* (pH2.3), and Ad5 *E1A* and *E1B* (pJOL). The level of *hsp70* gene transcription in exponentially growing control and heat shocked (15 min, 43°C) cell line 293 cells is shown (12-hr exposure). (*C*) Quantitation of the rates of *hsp70* gene transcription (○) and hsp70 mRNA levels (●) in serum-stimulated cells. The autoradiogram (*A*) was quantitated and normalized to the 18-hr level of hsp70 mRNA. The levels of ^{32}P-labeled RNAs (*B*) that hybridize to the *hsp70* gene were determined by liquid scintillation counting.

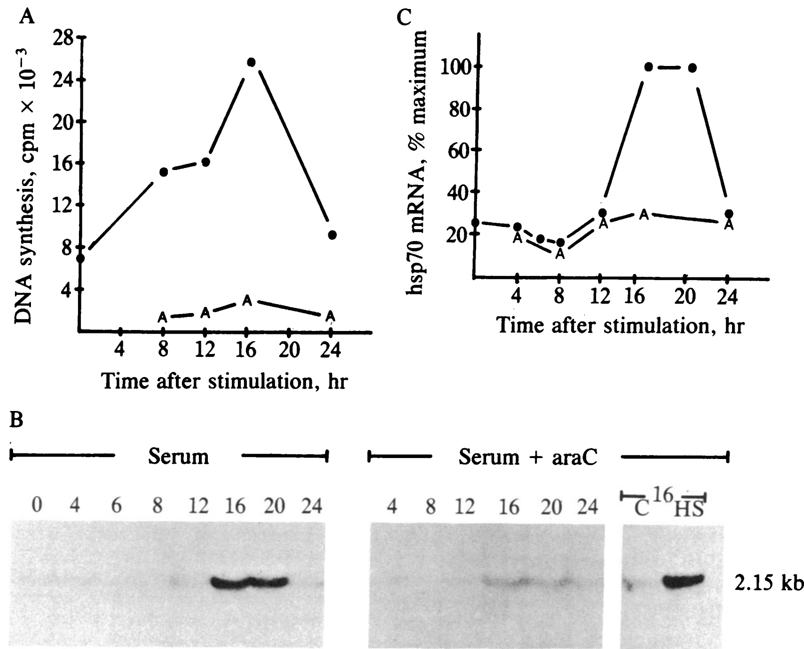

FIG. 4. Levels of hsp70 mRNA in serum-stimulated cell line 293 cells treated with araC. (*A*) DNA synthesis in serum-stimulated cell line 293 cells in the presence (A–A) or absence (●) of araC (20 μg/ml). (*B*) Relative levels of hsp70 mRNA at the indicated times after serum stimulation were determined by S1 nuclease protection. Cells treated for 16 hr with araC were heat shocked (HS) at 43°C for 1 hr and RNA was isolated for S1 nuclease protection. (*C*) Relative levels of hsp70 mRNA in serum-stimulated cell line 293 cells in the presence (A–A) or absence (●) of araC. The autoradiogram (*B*) was quantitated and the results were normalized.

also observed in the other six clonal lines. Therefore, the region upstream of the *hsp70* gene is likely to contain the regulatory sequences for serum-stimulated transcription.

DISCUSSION

The expression of the *hsp70* gene is serum-stimulated in two human cell lines. Transcription of the *hsp70* gene and ap-

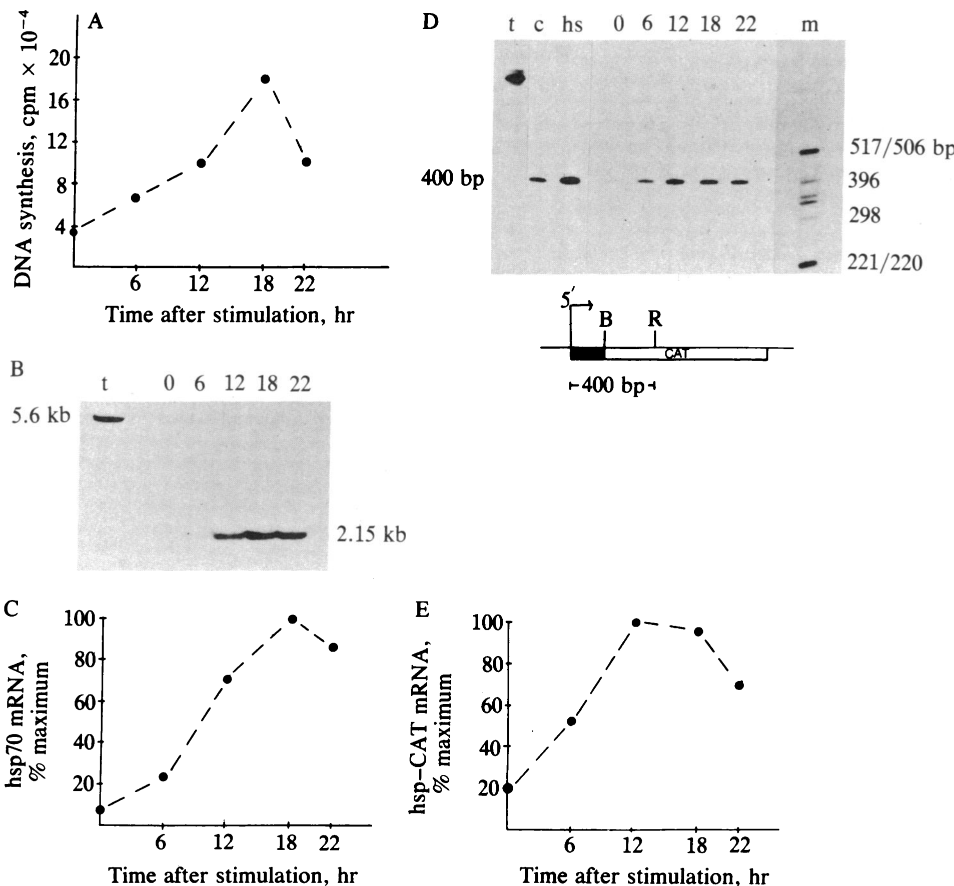

FIG. 5. Levels of hsp70 mRNA and chimeric pHB-CAT mRNA in serum-stimulated cell line 293 cells. (*A*) DNA synthesis ([^{3}H]thymidine incorporation) of 4 × 10^5 cells per point. (*B*) Relative levels of hsp70 mRNAs determined by S1 nuclease protection (12-hr exposure). (*C*) Quantitation of hsp70 mRNA levels. The autoradiogram (*B*) was quantitated and the results were normalized to the 18-hr level of hsp70 mRNA. (*D*) Relative levels of pHB-CAT mRNA determined by S1 nuclease protection. Cytoplasmic RNA from each point after serum stimulation was hybridized to a DNA template (t), pHBCAT, 5′-end-labeled at the *Eco*RI sites, analyzed by urea/acrylamide gel electrophoresis. Molecular length markers (m) are 3′-end-labeled *Hin*fI digests of pBR322. Scheme of the chimeric pHBCAT gene: arrow indicates direction of transcription from the 5′ terminus of the *hsp70* gene (solid area). bp, Base pairs; lane numbers indicate time (hr) after stimulation. (*E*) Quantitation of levels of pHB-CAT mRNA. The autoradiogram (*D*) was quantitated and the results were normalized to the 12-hr level of pHB-CAT mRNA.

pearance of cytoplasmic hsp70 mRNA increases in parallel with DNA synthesis. The serum-stimulated *hsp70* gene expression can be blocked by araC, an inhibitor of DNA synthesis.

The steady-state mRNA levels of several mammalian genes increase when cells are induced to proliferate. Expression of dihydrofolate reductase (24) and histone (25–30) genes are cell-cycle regulated; levels of c-myc mRNA remain invariant through the cell cycle (31), yet are responsive to mitogen induction (32). The temporal pattern and magnitude of serum-stimulated *hsp70* gene expression most closely resemble that for histone genes. There are, however, distinct features. While the periodic increase of histone mRNAs during serum stimulation or cell cycle has been attributed to message stability (25), posttranscriptional processing (26), or a combination of transcriptional activity and message stability (27, 28), the accumulation of hsp70 mRNA is primarily regulated at the level of transcription. Like histone genes, *hsp70* gene expression is coupled to DNA synthesis.

The human *hsp70* gene contains transcriptional regulatory sequences that are responsive to serum stimulation, Ad5 *E1A* and heat shock induction. The transcriptional activation of the human *hsp70* gene by three distinct inducers reveals the potential complexity in regulatory sequences and transcription factors. We have demonstrated that the *hsp70* gene remains heat shock inducible under conditions where the gene does not respond to serum stimulation. This suggests at least two distinct pathways for transcriptional regulation. Whether adenovirus *E1A* induction of the *hsp70* gene is facilitated by yet another mechanism remains to be tested.

The increased level of *hsp70* gene expression in cell line 293 cells is likely to be due to the expression of the integrated copies of Ad5 pre-early genes. This is supported by evidence that hsp70 is induced after Ad5 infection (14) and the *trans*-activation of viral and cellular genes by *E1A* (10, 11). If the serum-stimulated expression of the *hsp70* gene in cell line 293 cells is mediated and enhanced by *E1A*, then the similar response of HeLa cells to serum stimulation would suggest that HeLa cells contain a cellular analog of *E1A*. Alternatively, the serum-stimulated expression is independent of *E1A* activity because both human cell lines exhibit similar temporal patterns of *hsp70* gene expression. We note that transcription of sequences within the adenovirus pre-early region is increased in serum-starved cell line 293 cells and decreased at times of maximal hsp70 transcription. If, indeed, *E1A* activity is present in serum-starved cell line 293 cells, we suggest that *E1A* is not sufficient to induce hsp70 transcription.

The pattern of *hsp70* gene expression in serum-stimulated cells suggests a role for hsp70 during the synthetic phase of the cell cycle. This is of interest in view of the extensive homologies among the human and *Drosophila* hsp70 and *Escherichia coli* dnaK proteins (33, 34). Temperature-sensitive mutations in *E. coli* dnaK prevent both DNA and RNA synthesis at restrictive temperatures and block replication of bacteriophage λ (35, 36). The dnaK protein appears to be a component of the DNA replication machinery in *E. coli* (37). Similarly, mutations in yeast *hsp70* genes result in a temperature-sensitive growth phenotype (38). The temporal pattern of *hsp70* gene expression in human cells after serum stimulation is intriguing in view of the potential growth-related functions of the hsp70 homologues in yeast and *E. coli*. By analogy to the function of *E. coli* dnaK, the induced synthesis of hsp70 after adenovirus infection could signal the recruitment of macromolecules required for viral DNA replication.

We postulate that *hsp70* is essential for cell growth. The transcriptional activation of the *hsp70* gene by viral transforming genes (14–16) and a cellular rearranged oncogene (16) cannot be inconsequential. These oncogenes may disrupt the normal control of cell proliferation by affecting the transition from stage G_0 to G_1 of the cell cycle (32). The induction of *hsp70* may then be involved in cellular processes subsequent to the initiation of DNA synthesis, such as maintenance of the replication machinery.

We thank N. Jones, R. Mulligan, R. Kingston, and P. Sharp for providing recombinant plasmids; Eli Lilly for mycophenolic acid; and D. Linzer, P. Sharp, and C. Wu for their suggestions, encouragement, and comments on the manuscript. These studies were supported by grants from the Camille and Henry Dreyfus Foundation, the Leukemia Foundation, and the National Institutes of Health. B.J.W. is a fellow of the Leukemia Society of America.

1. Sambrook, J., Botchan, M., Gallimore, P., Ozanne, B., Pettersson, V., Williams, J. & Sharp, P. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 615–632.
2. Jones, N. & Shenk, T. (1979) *Cell* **17**, 683–689.
3. Rassoulzadegan, M., Naghashfar, Z., Cowie, A., Carr, A., Grisoni, M., Kamen, R. & Cuzin, F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4354–4358.
4. Houweling, A., Van den Elsen, P. & Van der Eb, A. (1980) *Virology* **122**, 109–124.
5. Ruley, H. E. (1983) *Nature (London)* **304**, 602–606.
6. Land, H., Parada, L. & Weinberg, R. (1983) *Nature (London)* **304**, 596–602.
7. Berk, A., Lee, F., Harrison, T., Williams, J. & Sharp, P. (1979) *Cell* **17**, 935–944.
8. Jones, N. & Shenk, T. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3665–3669.
9. Nevins, J. R. (1981) *Cell* **26**, 213–220.
10. Treisman, R., Green, M. & Maniatis, T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7428–7432.
11. Gaynor, R., Hillman, D. & Berk, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1193–1197.
12. Borreli, E., Hen, R. & Chambon, P. (1984) *Nature (London)* **312**, 608–615.
13. Velcich, A. & Ziff, E. (1985) *Cell* **40**, 705–716.
14. Nevins, J. R. (1982) *Cell* **29**, 913–919.
15. Imperiale, M. J., Kao, H.-T., Feldman, L. T., Nevins, J. R. & Strickland, S. (1984) *Mol. Cell. Biol.* **4**, 867–874.
16. Kingston, R., Baldwin, A., Jr., & Sharp, P. (1984) *Nature (London)* **312**, 280–282.
17. Wu, B., Hunt, C. & Morimoto, R. (1985) *Mol. Cell. Biol.* **5**, 330–341.
18. Graham, F., Smiley, J., Russell, W. & Nairn, R. (1977) *J. Gen. Virol.* **36**, 59–72.
19. Mulligan, R. & Berg, P. (1980) *Science* **209**, 1422–1427.
20. Weeks, D. & Jones, N. (1983) *Mol. Cell. Biol.* **3**, 1222–1234.
21. Banerji, S., Theordorakis, N. & Morimoto, R. (1984) *Mol. Cell. Biol.* **4**, 2437–2448.
22. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1373–1376.
23. Cozzarelli, N. (1977) *Annu. Rev. Biochem.* **46**, 641–668.
24. Wu, J.-S. & Johnson, L. (1982) *J. Cell. Physiol.* **110**, 183–189.
25. Stimac, E., Groppi, V., Jr., & Coffino, P. (1984) *Mol. Cell. Biol.* **4**, 2082–2090.
26. Alterman, R.-B., Ganguly, S., Schulze, D., Marzluff, W., Schildkraut, C. & Skoultchi, A. (1984) *Mol. Cell. Biol.* **4**, 123–132.
27. Heintz, N., Sive, H. & Roeder, R. (1983) *Mol. Cell. Biol.* **3**, 539–550.
28. Artishevsky, A., Delegeane, A. & Lee, A. (1984) *Mol. Cell. Biol.* **4**, 2364–2369.
29. Sive, H., Heintz, N. & Roeder, R. (1984) *Mol. Cell. Biol.* **4**, 2723–2734.
30. Graves, R. & Marzluff, W. (1984) *Mol. Cell. Biol.* **4**, 351–357.
31. Thompson, C. B., Challoner, P. B., Neiman, P. E. & Groudine, M. (1985) *Nature (London)* **314**, 363–366.
32. Kelly, K., Cochran, B., Stiles, C. & Leder, P. (1983) *Cell* **35**, 603–610.
33. Bardwell, J. & Craig, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 848–852.
34. Hunt, C. & Morimoto, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, in press.
35. Georgopoulas, C. P. (1977) *Mol. Gen. Genet.* **151**, 35–39.
36. Saito, H. & Uchida, H. (1978) *Mol. Gen. Genet.* **164**, 1–8.
37. Zylicz, M. & Georgopoulos, C. (1984) *J. Biol. Chem.* **259**, 8820–8825.
38. Craig, E. & Jacobsen, K. (1984) *Cell* **38**, 841–849.